Ticks and Tick-borne Diseases 10 (2019) 162–165

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis

Short Communication

Detection and molecular characterization of *Mogiana tick virus* (MGTV) in *Rhipicephalus microplus* collected from cattle in a savannah area, Uberlândia, Brazil

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ARTICLE INFO

Keywords:
Mogiana tick virus
Rhipicephalus microplus
Savannah Brazil
Virus
Bovine

ABSTRACT

In Brazil, recent studies have reported viruses detected in ticks with pathogenic potential in vertebrate hosts. Ticks of the species *Rhipicephalus microplus* collected from bovines in a savannah area were tested by RT-PCR for the presence of RNA targetting a segment of NS3-like protein gene of *Mogiana tick virus*, a member of the recently-described Jingmenvirus group. Amplification with size similar to the expected was observed with 25% (7/28) RNA samples of ticks that were collected from 39% (7/18) of the bovines. Nucleotide sequence analysis of three PCR products revealed divergence that varied from 3.3 to 5.0% in a single farm. Although *Jingmen tick virus*, another member belonging to the Jingmenvirus group, has been detected in human patients with Crimean-Congo hemorrhagic fever in Kosovo, whether or not MGTV causes disease in cattle and other animals remains to be investigated.

1. Introduction

Ticks are vectors of various microorganisms pathogenic to humans, household pets and wild animals (Jongejan and Uilenberg, 2004). Among them, viruses of three families, *Flaviviridae*, *Nairoviridae* and *Reoviridae*, have prominent importance in public health, particularly in Europe, Middle East, Asia and Africa (Lani et al., 2014).

In Brazil, studies on arboviruses have concentrated on those transmitted by mosquitoes (Figueiredo, 2007; Romano-Lieber and Iversson, 2000; Vasconcelos and Calisher, 2016) and reports about tick viruses are rare. Recently, however, two viruses isolated from ticks have been described. de Figueiredo et al. (2017) isolated *Cacipacoré virus* (CPCV), of the genus *Flavivirus* and family *Flaviviridae*, from a tick of the *Amblyomma cajennense* complex removed from a sick capybara in Matão, a rural area of São Paulo state. Maruyama et al. (2014) isolated another virus, named *Mogiana tick virus* (MGTV), initially thought to be a highly divergent *Flavivirus*, and detected in the cattle tick *Rhipicephalus microplus* collected from different regions of Brazil. A more detailed study revealed that MGTV is a member of a group of viruses named Jingmenvirus (Villa et al., 2017), which were described in 2014, and were detected in ticks of the same species collected in Jingmen, China (Qin et al., 2014).

Jingmen tick virus (JMTV) is a multi-segmented virus with four positive strand RNA segments, of which segments 1 and 3 contain coding sequences similar to the nonstructural (NS) proteins 5 and 2b-3, respectively, of flaviviruses (Holmes, 2016). Although evidence indicates that *Guaico Culex virus*, another member of the Jingmenvirus group, is a multicomponent virus (Ladner et al., 2016), whether or not all members of the group have their segments packaged in different virus particles remains unknown.

This work reports the molecular characterization of MGTV detected in *R. microplus* collected in 2015–2016 from cattle of Nelore breed (*Bos taurus indicus*) of Capim Branco farm, which is part of the Universidade Federal de Uberlândia, Uberlândia city, Minas Gerais state, southeastern Brazil. The property has a large area of native savannah, and the cattle are maintained in pasture adjoining natural vegetation. Cattle have sporadic contact with other livestock (sheep, goats and horses – respectively, *Ovis aries*, *Capra aegagrus hircus* and *Equus caballus*) and

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https://doi.org/10.1016/j.ttbdis.2018.10.002
Received 25 April 2018; Received in revised form 6 October 2018; Accepted 10 October 2018
Available online 13 October 2018

Table 1
Information on tick RNA samples tested in this study.

Collection date	Host/Sample ID[a]	Number of ticks per RNA sample	Tick(s) gender	RT-PCR result
07/31/2015	5	3	F	–
	50	1	F	–
	282	2	F	+
	296-a	2	F	–
	296-b	2	F	–
	296	1	M	–
	297	2	F	–
	307	2	F	+
	307	1	M	–
	375	2	F	+
05/31/2016	26	1	F	–
	313	2	F	+
	390	2	F	–
06/07/2016	287	2	F	–
	287	2	M	–
	407	2	F	–
	407	2	M	–
	1937-a	3	F	–
	1937-b	3	F	–
	1937	4	M	+
	7347	2	F	+
	7347	2	M	–
	8869	3	F	–
	8869	3	M	+
08/24/2016	36	2	F	–
	160	3	F	–
	181	1	F	–
	181	1	M	–

[a] If more than one tick was used in a sample, all ticks in the pool were collected from the same host and had the same gender.

also wild animals (giant anteater, collared anteater, capybara, puma, opossum, forest deer, and others – respectively, *Myrmecophaga tridactyla, Tamandua tretradactyla, Hydrochoerus hydrochaeris*, *Puma concolor*, *Didelphis marsupialis*, *Mazama gouazoubira*).

2. Material and methods

We collected 58 adult *R. microplus* ticks (40 females and 18 males; unfed and engorged ticks were used) from 18 bovines (Table 1). RNA was extracted with Trizol® (Life Technologies Corp/ThermoFisher Scientific. Grand Island, NY, USA) from individual ticks or from pools of two to four ticks (ticks were pooled according to host and gender as shown in Table 1, sliced, and homogenized with a pestle) following instructions of the manufacturer, but with half volume of all reagents. The detection of MGTV was performed by reverse transcription – polymerase chain reaction (RT-PCR) targetting a segment of NS3-like protein gene (a sequence of segment 3 of JMTV), with oligonucleotides 317-5-126 and 317-3-383 (GTT ACG GCT TCA GGA ACC AA and GGA GGG TTG CAT TTT TAG CA, respectively) described by Maruyama et al. (2014). Since amplification was not observed, for the purpose of improving the detection, a semi-nested PCR was carried out with primers 317-3-383 and FNAA (GGA ACC AAG GGG ATG ATA A, designed in this study based on the comparison of 43 partial sequences of MGTV reported by Maruyama et al., 2014). RT-PCR products were sequenced with ABI-Prism 3500 and BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems/ThermoFisher Scientific). SeqMan and MegAlign of Lasergene 15 (DNASTAR Inc. Madison, WI, USA) were used for editing, alignment, and phylogenetic analysis using Maximum Likelihood method. Bootstrap analysis was performed with 1000 replicates and the tree was obtained with Tree Explorer of MEGA7 (Kumar et al., 2016).

3. Results and discussion

Semi-nested PCR products of the expected size were observed with seven RNA samples (out of 28, 25%), five (out of 20, 25%) from females and two (out of eight, 25%) from males of *R. microplus* collected from seven bovines (out of 18), indicating a high prevalence (39%) of bovines with infected ticks. From four of these hosts, more than one tick RNA sample from the same host were tested and in all of them detection of MGTV RNA gave discordant results, with amplification observed in two female (host/sample IDs 307 and 7347) and two male (IDs 1937 and 8869) tick RNA samples, while their paired samples (from male and female ticks, respectively) were negative (Table 1).

Besides transmission by feeding on blood of infected hosts, arboviruses and *Rickettsia* have been reported to be transmitted from infected ticks to other ticks that are infesting the same host by co-feeding, even in seropositive hosts (Randolph, 2011; Wright et al., 2015; Zemtsova et al., 2010). Under experimental conditions using Balb/c mice, transmission of protopype *Tick-borne encephalitis virus* (TBEV) strain Hypr from infected to uninfected *Ixodes ricinus* ticks occurred via co-feeding, and the authors inferred that the result supported the importance of this transmission path for survival of the TBEV in nature (Slovák et al., 2014). In the present study, paired RNA samples were obtained from ticks collected on the same day and, even though the number of available samples was low, the discordant RT-PCR results suggest that MGTV may not be easily transmitted by either co-feeding or feeding on blood of the infected host, possibly due to the segmented nature of the virus.

Nucleotide sequence analysis of three PCR products revealed that, in a segment of 181 nucleotides, 12 synonymous substitutions were found, with 11 transitions (9 C/T, and 2 A/G) and one transversion (C/G) (Fig. 1). Sequence of sample 307 was the most divergent, with 95.0% identity (172/181) with the other samples, while identity between samples 282 and 7347 was 96.7% (175/181). The analysis carried out with other MGTV and JMTV sequences (Ladner et al., 2016; Maruyama

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Consensus CCCCCTCCACCCAAGCTCAGACCTGCCAGAGGAGAGGCAGGGTTGGAAGGAGAAAGGAGGGCCAGTATTATCGGCCAGCAAACTATGACCTGTTGCAAGC
          ---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+
                  10        20        30        40        50        60        70        80        90       100
          ---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+
 282      ...................................................................C................................
 307      ........................................................................................T..C........
 7347     .................................................A....................C.............................

Consensus CCCTCCCTTGGACCACCCCGACTCGGTCACGCTGCTGGAGGCCAACATGTGCCTTAGGGCACTCGGACTCCCCGAGGAGCC
          ---------+---------+---------+---------+---------+---------+---------+---------+-
                 110       120       130       140       150       160       170       180
          ---------+---------+---------+---------+---------+---------+---------+---------+-
 282      ...........................T......................................G..............
 307      ...C..T.....T.....T..............................................................
 7347     ..........................................T......................................
```

Fig. 1. Alignment of 181 nucleotide long sequence of segment 3 by sequencing of RT-PCR products obtained from tick RNA samples (282, 307, and 7347) collected from bovines in Uberlândia, Brazil. Consensus sequence is shown at the top, dots represent the residues that match the consensus and divergent residues are displayed.

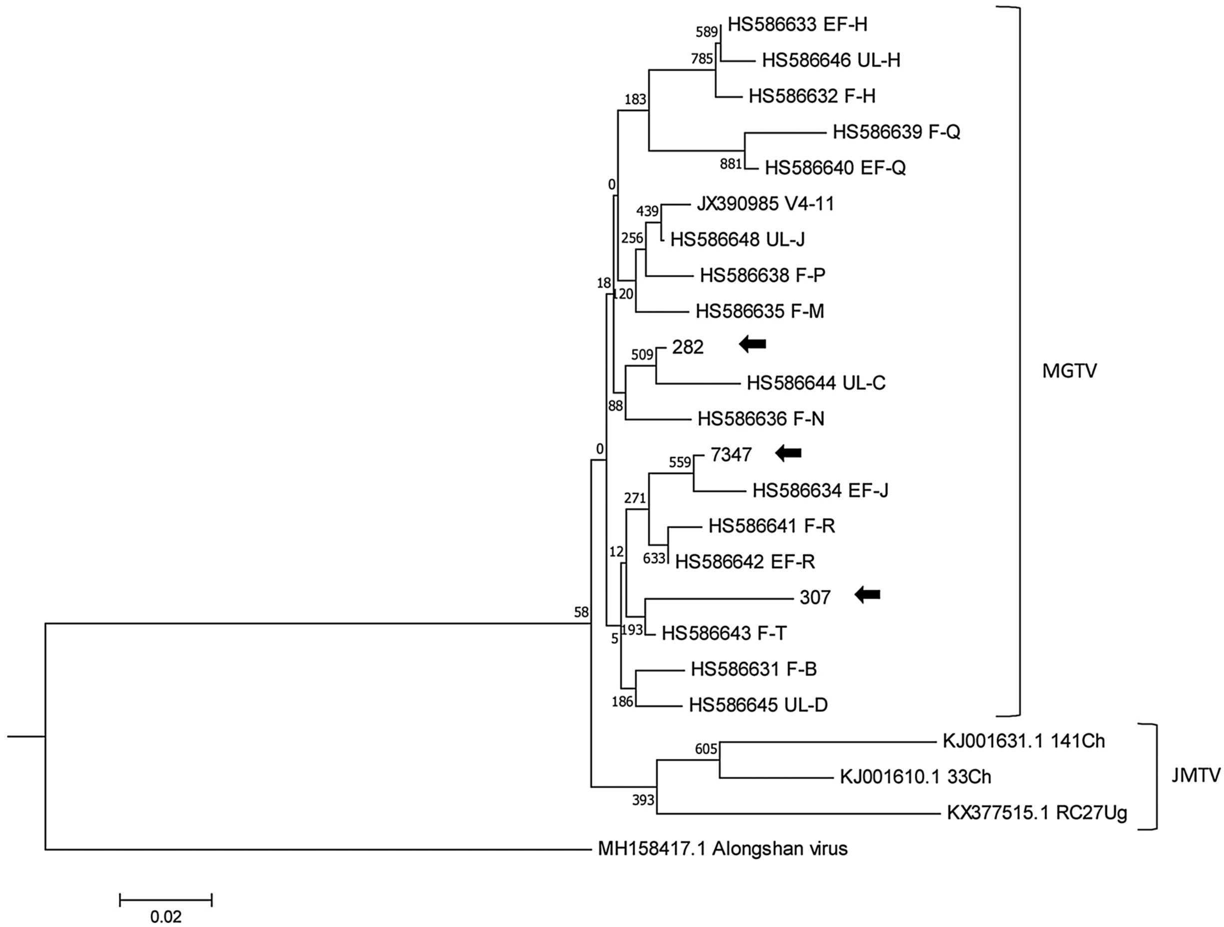

Fig. 2. Phylogenetic tree obtained by Maximum Likelihood method of 150 nucleotide long sequence of segment 3 of JMTV/MGTV isolates and of NS3-like protein gene of the *Flavivirus Alongshan virus* (accession numbers are shown). Bootstrap values (per 1000 replicates) are displayed at the nodes and the number of substitutions per site is indicated by the horizontal bar at the bottom. *Alongshan virus* sequence was selected as the outgroup for analysis based on higher nucleotide identity to MGTV (76.0 to 78.7%) compared to sequences of other flaviviruses (lower than 50.0%). Arrows indicate sequences 282, 307 and 7347 identified in this study. JMTV sequences were from China (Ch) and Uganda (Ug). The names of MGTV sequences used in the analysis followed Maruyama et al. (2014). UL, F, and EF represent, respectively, unfed larvae, female less than 4 mm in size (before engorgement phase of feeding), and engorged female; In Minas Gerais state – Q: Santa Vitória, C: Montes Claros, I: Uberlândia, J: Araguari, P: Pará de Minas; In Goiás state – D: Quirinópolis, L: Catalão, M: Piracanjuba, N: Itauçu; In Mato Grosso do Sul state: R: Cassilândia, S: Paranaíba, T: Água Clara, U: Ribas do Rio Pardo; In São Paulo state – A: Presidente Prudente, V: Birigui, X: Ribeirão Preto; In Rio de Janeiro state – B: Rezende; In Paraná or Rio Grande do Sul state: G and H; JX390985 contains the complete sequence of segment 3 of MGTV isolate V4-11.

et al., 2014) also revealed that nucleotide sequence of sample 7347 was closest (98.7% identity) to an MGTV sequence (EF-J) from ticks colleted in a region near Uberlândia (approximately 40 km away from the Capim Branco farm), while sequences of samples 282 and 307 were closest (98.0 and 96.7% identity, respectively) to MGTV sequences (UL-C and F-T, respectively) from ticks collected approximately 600 km away. Moreover, similar to the results observed by Ladner et al. (2016), MGTV sequences formed a clade that is distinct from the clades formed by JMTV sequences from China and from JMTV variant RC27 detected in plasma of a monkey in Uganda (Fig. 2) (respectively, Qin et al., 2014; Ladner et al., 2016). In regard to the deduced amino acid sequence, no substitution was observed compared to JMTV, except with variant RC27, which showed a unique substitution (glycine instead of alanine) in a 54 aa segment. The sequences 282, 307 and 7347 were deposited at GenBank and assigned nucleotide accession numbers MH033852, MH033853 and MH033854, respectively.

Uberlândia region has many farms with cattle, for meat and dairy production. Transporting cattle to and from different parts of Brazil for commercial reasons, as well as other countries, may function as a way of transporting ticks and the microorganisms that they carry. This may explain the sequence diversity we found in a single farm.

In the present work, ticks were collected from cattle that were maintained in an environment that is shared with wildlife. Furthermore, MGTV was first found in salivary glands of *R. microplus* and detected in both larvae and adults of this tick species (Maruyama et al., 2014). These observations may suggest that *R. microplus* may serve as a vector for transmission of MGTV to cattle and even to other animals, since this tick has been also found on horses. Although there is not yet any evidence that MGTV infects and replicates in cattle, of note, variant RC27 of JMTV was detected in plasma from a non-human primate in Uganda (Ladner et al., 2016), suggesting that JMTV (and possibly MGTV as well) may infect different host species. More recently, JMTV genome was detected in serum samples collected from three patients who presented Crimean-Congo hemorrhagic fever (CCHF) in Kosovo (Emmerich et al., 2018). Therefore, JMTV may have been transmitted to those patients by ticks of the genus *Hyalomma*, the primary vector of transmission of the virus that causes CCHF to humans (Whitehouse, 2004). In addition, many cattle on the farm were also infested by ticks of other species, such as *Amblyomma sculptum* of the *Amblyomma cajennense* complex (Siqueira, 2017), which also infests many other domestic and wild animals (Pereira et al., 2000; Szabó et al., 2001), and may transmit *Rickettsia* to humans. Whether or not MGTV causes disease in cattle and other animals remains to be elucidated.

Conflict of interest

None declared.

Acknowledgements

This research was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq (Academic Career Research Fellowship to M.P.J. Szabó). Tick collection in bovines agrees with ethical principles on animal research and approved by Ethics Commission on Use of Animals (CEUA) - Federal University of Uberlândia in 10/04/15 (protocol nº 010/15).

References

de Figueiredo, G.G., Amarilla, A.A., de Souza, W.M., Fumagalli, M.J., de Figueiredo, M.L.G., Szabó, M.P.J., Badra, S.J., Setoh, Y.X., Khromykh, A.A., Aquino, V.H., Figueiredo, L.T.M., 2017. Genetic characterization of *Cacipacoré virus* from ticks collected in São Paulo State, Brazil. Arch. Virol. 162, 1783–1786. https://doi.org/10.1007/s00705-017-3279-3.

Emmerich, P., Jakupi, X., von Possel, R., Berisha, L., Halili, B., Günther, S., Cadar, D., Ahmeti, S., Schmidt-Chanasit, J., 2018. Viral metagenomics, genetic and evolutionary characteristics of Crimean-Congo hemorrhagic fever orthonairovirus in humans, Kosovo. Infect. Genet. Evol. 65, 6–11. https://doi.org/10.1016/j.meegid.2018.07.010.

Figueiredo, L.T.M., 2007. Emergent arboviruses in Brazil. Rev. Soc. Bras. Med. Trop. 40, 224–229. https://doi.org/10.1590/S0037-86822007000200016.

Holmes, E.C., 2016. The expanding virosphere. Cell Host Microbe 20, 279–280. https://doi.org/10.1016/j.chom.2016.08.007.

Jongejan, F., Uilenberg, G., 2004. The global importance of ticks. Parasitology 129 (Suppl), S3–14. https://doi.org/10.1017/S0031182004005967.

Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33, 1870–1874. https://doi.org/10.1093/molbev/msw054.

Ladner, J.T., Wiley, M.R., Beitzel, B., et al., 2016. A multicomponent animal virus isolated from mosquitoes. Cell Host Microbe 20, 357–367. https://doi.org/10.1016/j.chom.2016.07.011.

Lani, R., Moghaddam, E., Haghani, A., Chang, L.Y., AbuBakar, S., Zandi, K., 2014. Tick-borne viruses: a review from the perspective of therapeutic approaches. Ticks Tick Borne Dis. 5, 457–465. https://doi.org/10.1016/j.ttbdis.2014.04.001.

Maruyama, S.R., Castro-Jorge, L.A., Ribeiro, J.M., Gardinassi, L.G., Garcia, G.R., Brandão, L.G., Rodrigues, A.R., Okada, M.I., Abrão, E.P., Ferreira, B.R., Fonseca, B.A., Miranda-Santos, I.K., 2014. Characterisation of divergent flavivirus NS3 and NS5 protein sequences detected in *Rhipicephalus microplus* ticks from Brazil. Mem. Inst. Oswaldo Cruz 109, 38–50. https://doi.org/10.1590/0074-0276130166.

Pereira, M., de, C., Szabó, M.P., Bechara, G.H., Matushima, E.R., Duarte, J.M., Rechav, Y., Fielden, L., Keirans, J.E., 2000. Ticks (Acari: Ixodidae) associated with wild animals in the Pantanal region of Brazil. J. Med. Entomol. 37, 979–983. https://doi.org/10.1603/0022-2585-37.6.979.

Qin, X.-C., Shi, M., Tian, J.-H., Lin, X.-D., Gao, D.-Y., He, J.-R., Wang, J.-B., Li, C.-X., Kang, Y.-J., Yu, B., Zhou, D.-J., Xu, J., Plyusnin, A., Holmes, E.C., Zhang, Y.-Z., 2014. A tick-borne segmented RNA virus contains genome segments derived from unsegmented viral ancestors. Proc. Natl. Acad. Sci. U. S. A. 111, 6744–6749. https://doi.org/10.1073/pnas.1324194111.

Randolph, S.E., 2011. Transmission of tick-borne pathogens between co-feeding ticks: Milan Labuda's enduring paradigm. Ticks Tick Borne Dis. 2, 179–182. https://doi.org/10.1016/j.ttbdis.2011.07.004.

Romano-Lieber, N.S., Iversson, L.B., 2000. Serological survey on arbovirus infection in residents of ecological reserve. Rev. Saúde Pública 34, 236–242. https://doi.org/10.1590/S0034-89102000000300005.

Siqueira, S.M., 2017. Fluxo de carrapatos entre ambiente e animais na fazenda Capim Branco, Uberlândia-MG: considerações epidemiológicas. Doctoral dissertation in Veterinary Sciences – Faculdade de Medicina Veterinária, Universidade Federal de Uberlândia, Uberlândia, Brazil, pp. 122. https://doi.org/10.14393/ufu.te.2018.27.

Slovák, M., Kazimírová, M., Siebenstichová, M., Ustaníková, K., Klempa, B., Gritsun, T., Gould, E.A., Nuttall, P.A., 2014. Survival dynamics of tick-borne encephalitis virus in *Ixodes ricinus* ticks. Ticks Tick Borne Dis. 5, 962–969. https://doi.org/10.1016/j.ttbdis.2014.07.019.

Szabó, M.P., Cunha, T.M., Pinter, A., Vicentini, F., 2001. Ticks (Acari: Ixodidae) associated with domestic dogs in Franca region, São Paulo, Brazil. Exp. Appl. Acarol. 25, 909–916. https://doi.org/10.1023/A:1020433003028.

Vasconcelos, P.F., Calisher, C.H., 2016. Emergence of human arboviral diseases in the Americas, 2000–2016. Vector-Borne Zoonotic Dis. 16, 295–301. https://doi.org/10.1089/vbz.2016.1952.

Villa, E.C., Maruyama, S.R., de Miranda-Santos, I.K.F., Palacios, G., Ladner, J.T., 2017. Complete coding genome sequence for *Mogiana tick virus*, a jingmenvirus isolated from ticks in Brazil. Genome Announc. 5, e00232–17. https://doi.org/10.1128/genomeA.00232-17.

Whitehouse, C.A., 2004. Crimean-Congo hemorrhagic fever. Antiviral Res. 64, 145–160. https://doi.org/10.1016/j.antiviral.2004.08.001.

Wright, C.L., Sonenshine, D.E., Gaff, H.D., Hynes, W.L., 2015. *Rickettsia parkeri* transmission to *Amblyomma americanum* by cofeeding with *Amblyomma maculatum* (Acari: Ixodidae) and potential for spillover. J. Med. Entomol. 52, 1090–1095. https://doi.org/10.1093/jme/tjv086.

Zemtsova, G., Killmaster, L.F., Mumcuoglu, K.Y., Levin, M.L., 2010. Co-feeding as a route for transmission of *Rickettsia conorii israelensis* between *Rhipicephalus sanguineus* ticks. Exp. Appl. Acarol. 52, 383–392. https://doi.org/10.1007/s10493-010-9375-7.